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enzyme complex is comprised of components of mouse and human origin, including mTERT encoded by the cDNA of SEQ ID NO:1 and an hTERC moiety. The mouse telomerase enzyme-associated RNA component (mTERC) has been cloned and characterized, see USSN 08/782,787, filed 10 February 1997; 08/670,516, filed 27 June 1996 (now abandoned); and 08/485,778, filed 7 June 1995 (now US 5876979). In addition, hTERC (hTR) knockout mice have been constructed, see USSN 08/623,166, filed 28 March 1996 (now abandoned). hTERC has been cloned and characterized, see PCT Publication Nos. 96/01835 and 96/40868 and U.S. Patent No. 5,583,016.

In alternative embodiments the telomerase can include any number of enzyme complex-associated proteins, such as co-purifying proteins and other proteins that regulate enzyme activity.

The present invention provides a number of different methods for expressing and isolating mTERT, telomerase enzyme and telomerase-associated compounds that can be employed, in one or more aspects, as reagents and are useful in methodologies as described herein. The novel reagents and methods of the invention provide for mice lacking in full or partial mTERT activity, i.e., mTERT "knockout" mice, and methods for making such mice.

The telomerase-associated protein can be, for example, the mouse homologue of the *Tetrahymena* p80 protein, described in Harrington (1997) *Science* 275:973. While some telomerase-associated proteins are known, the present invention provides methods and reagents for identifying additional telomerase enzyme-associated proteins and other compounds and assembling (*i.e*, "reconstituting") them with mTERT. Such telomerase enzyme-associated proteins can be prepared in accordance with, *e.g.*, USSN 08/883,377 (now US.5888910) and PCT application No. 97/06012, both filed April 4, 1997; and PCT application No. 96/14679, USSN 08/710,249 (now US 5858777) and 08/713,922 (now abandoned), all filed September 13, 1996. The *Tetrahymena* p80 and p95 putative telomerase proteins are described in PCT publication No. 96/19580.

The invention, providing for mTERT isoforms, homologues and alleles, describes structural features common to the mTERT species of the invention in the form of structural motifs, see Figures 4 and 5. These motifs can effect common mTERT and telomerase enzyme functions. Sequence analysis of mTERT shows that it contains murine-specific amino acid regions, *i.e.*, "motifs," common to other mTERT proteins, as illustrated in Figure 5.

Novel reagents of the invention corresponding to these motif regions can be used in methods of the invention to generate antibodies and to identify additional mTERT isoforms, homologues and alleles. The invention provides oligonucleotides corresponding to these motif regions, including restriction enzyme fragments and amplification products generated from an mTERT. Oligonucleotides corresponding to motifs can also be synthesized *in vitro*. These oligonucleotides can also be used as PCR amplification primers or hybridization probes to identify and isolate additional mouse isoforms, homologues and alleles. These oligonucleotides can also be used as primers to amplify additional mTERT species, using techniques such as RACE, as described below.

The invention further provides for an isolated, purified or recombinant mouse telomerase enzyme complex capable of replicating telomeric DNA or any sequence determined by a telomerase enzyme-associated nucleic acid component. The telomerase enzyme complex of the invention can comprise components that are purified or isolated from a natural or synthetic source, a recombinantly manufactured.

The mTERT of the complex can be modified to delete the full or a "partial activity" of the TERT or enzyme complex, as described below.

Telomerase reverse transcriptase enzymes and mTERT are very rare in nature, and few successful attempts have been made to purify the enzyme complex; see, as examples of such successful purification, USSN 08/510,736, filed August 4, 1995, now abandoned and USSN 08/833,377, now US 5968506, and PCT application No. 97/06012, both filed April 4, 1997. The aforementioned patent applications provide useful methodologies and reagents that can be applied to the methods and reagents of the present invention. The present invention provides a variety of methods and reagents for creating the most pure mouse telomerase enzyme and mTERT preparations ever made, including methods for making recombinant telomerase enzyme and mTERT in abundant levels in recombinant host cells, methods for producing telomerase enzyme and mTERT synthetically and in cell-free translation systems. The invention provides methods for isolating recombinant or native telomerase enzyme, mTERT and telomerase components by reacting the telomerase or mTERT with an anti-telomerase antibody of the invention.

Also provided are methods and compositions for the expression of the mouse telomerase enzymes and mTERTs of the invention. In alternative embodiments, the

compositions of the invention are expressed as fusion proteins comprising exogenous sequences to aid in cell targeting, purification, expression and/or detection of mTERT and telomerase enzyme. The recombinant telomerase enzyme, mTERTs and telomerase-associated compositions of the invention can be independently or co-expressed in any system, including bacteria, yeast, fungi, insect or mammalian cells or the whole organism. The telomerase enzymes and mTERTs of the invention can also be expressed *ex vivo*, or *in vivo*, *e.g.*, as in transgenic non-human animals.

The invention also provides for methods of reconstituting telomerase enzyme and mTERT activity, including full and partial activity, *in vitro* and *in vivo*, using the purified mTERT of the invention, with or without further incorporation of its RNA moiety or telomerase-associated components. As used herein, the term reconstitution of a telomerase activity in a cell or animal also includes inducement, augmentation or replacement of low, lost or "knocked out" telomerase enzyme or mTERT activity. In one embodiment, the method can reconstitute "full" telomerase activity, *i.e.*, the ability to synthesize telomere DNA. Alternatively, the reconstitution can be only for "partial activities," as described in detail below. The invention include reconstitution of hTERT in such mTERT "knockout" mice, and the animals and their progeny produced by such reconstitution. The cloning and characterization of hTERT is described, *e.g.*, in USSN 08/854,050, filed May 9, 1997; in USSN 08/915,503, USSN 08/912,951, and, USSN 08/911,312, all filed August 14, 1997; and in USSN 08/974,549, and USSN 08/974,584, both filed on November 19, 1997.

The assays of the invention can be used to assess the degree of purification, identify a new mTERT species, such as an mTERT allele, homologue, or isoform, or to screen for modulators (antagonists and agonists) of telomerase-mediated DNA replication. Methods for identifying modulators of a telomerase enzyme activity have been described in U.S. Patent No. 5,645,986; and USSN 08/151,477, filed November 12, 1993; and USSN 08/288,501, filed August 10, 1994, and the reagents of the invention may be employed in such methods. Antagonists and agonists of mTERT can be used to modify the activity of other telomerase enzymes, such as hTERT (hTRT).

The invention contemplates screening for compositions capable of modifying the polymerase activity of telomerase enzyme, or a partial activity, by any means. In various embodiments, the invention includes: screening for antagonists that bind to mTERT's active

site or interfere with transcription of its RNA moiety, as mTERC; screening for compositions that inhibit the association of nucleic acid and/or telomerase-associated compositions, such as the association of mTERC with mTERT or the association of mTERT with mouse p80-homologue or other telomerase-associated proteins, or association of mTERT with a telomere, chromosome, nucleosome or a nucleotide; screening for compositions that promote the dissociation or promote the association of the enzyme complex, such as an antibody directed to mTERC or mTERT; screening for agents that effect the processivity of the enzyme; and screening for nucleic acids and other compositions that bind to mTERT, such as a nucleic acid complementary to mTERC. The invention further contemplates screening for compositions that increase or decrease the transcription of the mTERT gene and/or translation of the mTERT gene product. These compositions can be used to modify the transcription or translation of other TERT genes, such as hTERT.

Screening for antagonist activity provides for compositions that decrease telomerase replicative capacity, thereby limiting the proliferative, replicative potential of indefinitely proliferating cells, or mortalizing otherwise immortal cells, such as cancer cells.

Screening for agonist activity or transcriptional or translational activators provides for compositions that increase the telomerase enzyme's telomere replicative capacity, or, alternatively, a partial activity as described herein. Such agonist compositions provide for methods of creating indefinitely proliferating cells, and immortalizing or increasing the proliferative capacity of otherwise normal, untransformed cells, including cells which can express useful proteins. Such agonists can also provide for methods of controlling cellular senescence, see co-pending USSN 08/912,951 and 08/915,503.

now US 6475789 now abandoned

The novel telomerase compositions and activity reconstitution assays of the invention also provide for a novel telomerase repeat amplification protocol assay (TRAP) and variations of this assay. The TRAP assay is an amplification-based method for detecting, determining, and measuring telomerase activity and is described in PCT Publication Nos. 97/15687 and 95/13381 and U.S. Patent No. 5,629,154; see also USSN 08/632,662, and USSN 08/631,554, filed 15 April 1996 and 12 April 1996, respectively. See also, Kim (1994) *supra.* The present invention provides reagents useful for the TRAP assay as well as new amplification-based telomerase activity assays for a wide variety of applications. For example, TRAP assays comprising an mTERT protein or a telomerase enzyme complex of the

now US 5804380 now 5863726

invention can be used to screen for modulators of telomerase activity. Such compositions can also be used to modulate the activity of other telomerase enzymes, such as hTERT, or to act as a basis for identification of such human telomerase enzyme modulators.

The novel telomerase compositions of the invention can also be used in telomere length assays. Because of the relationship between telomerase activity and telomere length, the diagnostic and therapeutic methods of the invention can be used in conjunction with telomere length assays. A variety of telomere length assays have been described, see PCT Patent publication Nos. 93/23572, 95/13382, 95/13383, and 96/41016, and USSN 08/660,402, filed 6 June 1996; 08/479,916, filed June 7, 1995; and, 08/475,778 and 08/487,290, both filed June 7, 1995.

The invention provides a method of screening for telomerase modulators in animals by reconstituting a telomerase activity, or an anti-telomerase activity, into an animal, such as a transgenic, non-human animal. The invention provides for *in vivo* assays systems that include mouse "knockout" models in which the endogenous mTERT has been deleted, altered, or inhibited. The endogenous mTERT can be deleted, altered, or inhibited in either one or both endogenous mTERT alleles. One embodiment provides for a telomerase deficient mouse, or mTERT "knockout" mouse, and its progeny. Other embodiments provide for "knockout" mice, and their progeny, whose ability to express the telomerase RNA moiety and/or telomerase-associated proteins has also been deleted, altered, or modified. In one embodiment, an exogenous telomerase activity (such as human TERT), or endogenous mouse telomerase activity, full or partial, wild-type or modified, is reconstituted in the "knock-out" mouse or increased in an otherwise normal mouse. In alternative embodiments, endogenous mouse telomerase enzyme or mTERT activities, full or partial, can remain either in one or both alleles. The telomerase activity reconstituted in the "knockout" mouse model can include modified endogenous or exogenous TERT, *e.g.*, mTERT or hTERT alone, hTERT and hTERC, mTERT and mTERC, mTERT and hTERC. The invention also provides for transgenic cells and animals, in addition to mice, where mTERT and/or murine telomerase activity has been inserted through recombinant methodologies. The non-human transgenic animals of the invention also provide for methods of expressing large amounts of fully or partially active telomerase enzyme and mTERT. Transgenic animals also provide for the

that the cells may lose growth control and change to a state of uncontrolled cell growth, becoming a cancer, tumor or other malignancy. The present invention solves this complication by providing means to express mTERT or other telomerase components conditionally and/or by providing means for knocking out telomerase enzyme, mTERT or a telomerase enzyme complex component necessary for activity. Moreover, even "mortal" cells used in transplantation or for other purposes can be mortalized by such methods of the invention. Without an active telomerase, the cells are irreversibly mortal, thus decreasing the probability of cancerous or malignant transformation after transplantation or other re-introduction into a host organism. This would not affect the cell's function, as telomerase enzyme is not normally active in somatic cells.

The present invention also provides methods and reagents relating to *cis*-acting transcriptional and translational regulatory elements. Examples of *cis*-acting transcriptional regulatory elements include promoters and enhancers of the mTERT gene. Examples of *cis*-acting translational regulatory elements include elements that stabilize mRNA or protect the transcript from degradation. The identification and isolation of *cis*- and *trans*-acting regulatory agents provide for further methods and reagents for identifying agents that modulate transcription and translation of mTERT and other telomerase enzymes and TERTs, such as hTERT. While many aspects of these methods and reagents are described more fully below, USSN 08/714,482, filed September 16, 1996, now US 5972605 provides useful information relating to reagents and screens for the hTERC (hTR) promoter that usefully supplements understanding of certain embodiments of the present invention relating to the mTERC promoter and isolated and recombinant molecules comprising all or part of the mTERT promoter and related methods.

The present invention also provides novel methods and reagents for immunizing animals to generate an anti-murine telomerase enzyme and an anti-mTERT immune response. While these methods and compositions are fully described below, see also USSN 08/734,052, filed October 18, 1996, now abandoned for additional useful information.

1. NUCLEIC ACIDS ENCODING mTERTs

This invention for the first time provides the identification, cloning and characterization of the mTERT gene, related polypeptide, and telomerase enzyme complexes

The program can also be used to plot a dendogram or tree representation of clustering relationships. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison. For example, hTERT can be compared to other TERT species using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps.

Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul (1990) *J. Mol. Biol.* 215: 403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, ~~http://www.ncbi.nlm.nih.gov/~~ ; see also Zhang (1997) *Genome Res.* 7:649-656 (1997) for the "PowerBLAST" variation. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al, supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (*see* Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLAST algorithm performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

variations or mutated sequences where the PCR or LCR primers or other reagents are designed to be extended or ligated only when a specific sequence is present. Alternatively, the specific sequences can be generally amplified using, for example, more generic PCR primers and the amplified target region later probed or sequenced to identify a specific sequence indicative of the allele or mutation.

It will be appreciated that nucleic acid hybridization assays for identification, diagnosis, sequencing, and the like, of mTERT can also be performed in an array-based format. Arrays involve a multiplicity of different "probe" or "target" nucleic acids (or other compounds) that are hybridized against a target nucleic acid. In this manner a large number of different hybridization reactions can be run essentially "in parallel". This provides rapid, essentially simultaneous, evaluation of a wide number of reactants. Methods of performing hybridization reactions in array based formats are well known to those of skill in the art, *e.g.*, Jackson (1996) *Nature Biotechnology* 14:1685; Chee, *Science* 274:610 (1995); Pastinen (1997) *Genome Res.* 7:606-614, describing minisequencing on oligonucleotide arrays; and Drobyshev (1997) *Gene* 188:45-52, for sequence analysis by hybridization with oligonucleotide microchip.

An alternative means for determining the level of expression of a gene encoding a protein is *in situ* hybridization. *In situ* hybridization assays are well known and are generally described in Angerer (1987) *Methods Enzymol* 152:649. In an *in situ* hybridization assay, cells can be fixed to a solid support, typically a glass slide, or be free in solution. If DNA is to be probed, the cells are typically denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of labeled probes specific to the nucleic acid sequence encoding the protein. The probes are typically labeled, *i.e.*, with radioisotopes or fluorescent reporters. See also U.S. Patent No. 5,583,016, USSNs 08/472,802 and 08/482,115, both filed June 7, 1995; USSN 08/521,634, filed August 31, 1995; USSN 08/714,482, filed Sept 16, 1996; and USSNs 08/770,564 and 08/770,565, both filed 20 December 1996; Soder (1997) *Oncogene* 14:1013-1021, all of which describe *in situ* hybridization of hTERC. Another well-known *in situ* hybridization technique is the so-called FISH fluorescence *in situ* hybridization, see Macechko (1997) *J. Histochem. Cytochem.* 45:359-363; and Raap (1995) *Hum. Mol. Genet.* 4:529-534.

specification). General methods for producing the antibodies of the invention are described below.

Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art and described in the scientific and patent literature, see, *e.g.*, Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) *Nature* 256:495; Harlow and Lane (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Such techniques include selection of antibodies from libraries of recombinant antibodies displayed in phage ("phage display libraries") or similar on cells. *See*, Huse (1989) *Science* 246:1275; Ward (1989) *Nature* 341:544; Hoogenboom (1997) *Trends Biotechnol.* 15:62-70; Katz (1997) *Annu. Rev. Biophys. Biomol. Struct.* 26:27-45. Recombinant antibodies can be expressed by transient or stable expression vectors in mammalian cells, as in Norderhaug (1997) *J. Immunol. Methods* 204:77-87; or in yeast, Boder (1997) *Nat. Biotechnol.* 15:553-557.

To produce large amounts of antibodies for use in, for example, immunoaffinity purification or diagnostics, a number of immunogens provided by the invention may be used. Telomerase enzyme or mTERT isolated or purified from a natural source (see co-pending USSN 08/833,377, filed April 4, 1997), now US 5968506 from a recombinant protein isolated from transformed cells provided by the present invention, or isolated as a synthetically produced composition, can be used as immunogens for the production of monoclonal or polyclonal antibodies. Naturally occurring murine telomerase enzyme or mTERT proteins or recombinant mTERT and/or telomerase enzyme can be used either in pure or impure form. Synthetic peptides are made using any portion of the mTERT amino acid sequence for use as immunogens, particularly peptides comprising the motif structures described herein. The peptides can be used alone or conjugated to another composition as immunogens.

Methods for the production of polyclonal and monoclonal antibodies are known to those of skill in the art. In brief, an immunogen is mixed with an adjuvant, as described above, and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the

buffer (*e.g.*, 20 mM sodium phosphate, pH 6.8, 150 mM NaCl). Other appropriate buffers will be apparent to those of skill in the art. Following the washing step, the inclusion bodies can be solubilized by the addition of a solvent that is both a strong hydrogen acceptor and a strong hydrogen donor (or a combination of solvents each having one of these properties) together with a reducing agent such as DTT. The proteins that formed the inclusion bodies can then be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to, urea (typically from about 4 M to about 8 M), formamide (typically at least about 80%, volume/volume basis), and guanidine hydrochloride (typically from about 4 M to about 8 M). Some solvents capable of solubilizing aggregate-forming proteins include, *e.g.*, SDS (sodium dodecyl sulfate), 70% formic acid, but may be inappropriate if irreversible denaturation of the proteins occurs, which is typically accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant, allowing re-formation of immunologically and/or biologically active protein. The protein can be separated during or after solubilization from other bacterial or other contaminating host proteins by standard separation techniques using the reagents of the invention in accordance with the methods of the invention.

iv. Standard Protein Separation Techniques

The present invention can provides methods for purifying telomerase enzyme and mTERT from a natural source or as a recombinant protein from transformed cells or transgenic animals. The novel reagents of the invention, such as the anti-mTERT antibodies, can be used to improve purification procedures, such as those described in co-pending USSN 08/833,377, filed April 4, 1997. Some illustrative examples of methods for purifying murine telomerase enzyme, mTERT, and other compositions used in the methods of the invention are described below.

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(1) Solubility Fractionation

If the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely

i. Dot Blot Assay

Another assay for telomerase activity is the dot blot assay. The dot blot assay is useful for routine screening because it can be used in high throughput mode, and hundreds of assays can be carried out in a single day with a good portion of the labor performed automatically. The dot blot assay is most effective for comparing activity of samples at roughly the same level of purity and is less effective for a multiplicity of samples at different stages of purity, and so may not be a preferred assay for determining relative purity. See co-pending USSN 08/833,377, filed April 4, 1997. now US 5 968 506

ii. Reverse Transcription PCR/ Quantitative PCR

The present invention provides polymerase chain reaction (PCR) assays that can be used to detect and quantify levels of telomerase enzyme-generated product. See also, U.S. Patent No. 5,629,154. Other target amplification techniques can also be employed in these methods, and one of skill in the art will appreciate that, whatever amplification method is used, if a quantitative result is desired, care must be taken to use a method that maintains or controls for the relative amplification of the various nucleic acids amplified. PCR is discussed in general above. a comprehensive discussion on quantitative PCR can be found in the scientific and patent literature, and is, for example, outlined in Innis, *supra*; see also Okamoto (1997) *Biol. Pharm. Bull.* 20:1013-1016.

iii. Telomeric Repeat Amplification Protocol (TRAP Assay)

The invention also provides for novel embodiments of the TRAP assay and variations of this well known telomerase activity assay. The present invention provides reagents useful for the TRAP assay as well as new amplification based telomerase activity assays for a wide variety of applications.

One limitation of the primer extension assay, described above, for assessing telomerase activity is weak signal strength, often necessitating long (7 or more days) autoradiographic exposure. Fortunately, the highly sensitive PCR-based "TRAP" assay for measuring telomerase activity has been developed. The TRAP assay is an amplification-based method for detecting, determining, and measuring telomerase activity and is described in PCT Publication Nos. WO 97/15687 and WO 95/13381 and U.S. Patent No. 5,629,154; see also USSN 08/632,662, now US 5804380 and USSN 08/631,554, now US 5863726 filed 15 April 1996 and 12 April 1996, respectively. See also, Kim (1994) *Science* 266:2011; PCT/US96/09669; Piatyszek (1995)

polyanionic heparin (Bock (1992) *Nature* 355:564-566). Because mTERT protein binds both mTERC (or hTERC) and its DNA substrate, and because the present invention provides mTERT and other mTERT-associated proteins in isolated and purified forms in large quantities, those of skill in the art can readily screen for mTERT-binding aptamers using the methods of the invention.

Antagonists of telomerase-mediated DNA replication can also be based on inhibition of mTERC (Norton (1996) *Nature Biotechnology* 14:615-619) through complementary sequence recognition or cleavage, as through ribozymes.

Telomerase activity can be inhibited by targeting mTERT mRNA with antisense oligonucleotides capable of binding mTERT mRNA. In some situations, naturally occurring nucleic acids used as antisense oligonucleotides may need to be relatively long (18 to 40 nucleotides) and present at high concentrations. a wide variety of synthetic, non-naturally occurring nucleotide and nucleic acid analogues are known which can address this potential problem. For example, peptide nucleic acids (PNAs) containing non-ionic backbones, such as N-(2-aminoethyl) glycine units can be used. PNAs targeting hTERC have been described, as well as methods for internalizing such PNAs in cells. See, USSN 08/630,019, filed April 9, 1996, and USSN 08/838,545 and PCT/US/97/05931, filed on April 9, 1997 (also, see Norton (1996) *supra*). Antisense oligonucleotides having phosphorothioate linkages can also be used, as described in WO 97/03211; WO 96/39154; Mata (1997) *Toxicol Appl Pharmacol* 144:189-197; Antisense Therapeutics, ed. Sudhir Agrawal (Humana Press, Totowa, New Jersey, 1996). Antisense oligonucleotides having synthetic DNA backbone analogues provided by the invention can also include phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, and morpholino carbamate nucleic acids, and other synthetic, non-naturally occurring nucleotide and oligonucleotide mimetics.

As noted above, combinatorial chemistry methodology can be used to create vast numbers of oligonucleotides that can be rapidly screened for specific oligonucleotides that have appropriate binding affinities and specificities toward any target, such as the mTERT proteins of the invention, can be utilized (see Gold (1995) *J. of Biol. Chem.* 270:13581-13584).

made using a 3' oligo-dT primer in a 3' RACE amplification protocol, as generally described above. Subsequently, the primers mTRT.10 (5'-CGTCGATACTGGCAGATGCGG-3') (SEQ ID NO:13) and mTRT.53 (5'-GTGCTGAGGCTACAATGCCCATGT-3') (SEQ ID NO:14) were amplified at 94°C for 30 min., 68°C for 3 min., for 30 cycles; followed by 30 more cycles using primers mTRT.9 (5'-CTTTTACATCACAGAGAGCAC-3') (SEQ ID NO:15) and mTRT.52 (5'-CATGTTCATCTAGCGGAAGGAGACA-3') (SEQ ID NO:16) . The PCR product (called mTRT Ra-52) was cloned into pCR II (Invitrogen, San Diego,CA), and 5 independent clones were isolated and the mTERT inserts sequenced (called mTRT Ra52). The DNA insert sequence was identical for all 5 clones and matched the sequence of the mTERT PCR amplification products described above, including the entire mTERT open reading frame. A unique NheI restriction site located in the region of the overlap between this RT-PCR product (called mTRT Ra 52.17 or pGRN189) and the 5' mTERT cDNA clone was utilized to construct the full length mTERT ORF. The plasmid with this full-length ORF was designated pGRN188. ~~pGRN188 has been submitted to ATCC, given the Accession No. ATCC _____~~. The mTERT insert of pGRN188 (SEQ ID NO:1) has been submitted to Genbank as Accession No. AF051911 (and is incorporated by reference herein, as noted below).

Figure 1 shows the complete sequence of the mTERT cDNA (SEQ ID NO:1). Figure 2 shows the deduced translation (polypeptide) product (SEQ ID NO:2). Figure 6 shows a preliminary sequencing of the genomic promoter region of mTERT (SEQ ID NO:4).

Cloning and Sequencing of Genomic mTERT DNA

A lambda phage (called lambda-mTERT1) with an approximately 23 kilobase pair (Kbp) insert containing the ATG initiator for mTERT was cloned from a mouse genomic 129SV phage library (Stratagene, San Diego, CA) using a mTERT cDNA probe (residues 1586 to 1970 from SEQ ID NO:1). Two subfragments of lambda-mTERT1 (an 8 Kbp HindIII and a 6 Kbp BglII fragment) were found to hybridize to portions of pGRN227 in a Southern hybridization experiment, see map, Figure 7. The 8kb HindIII phage DNA fragment was subcloned into the HindIII site of Bluescript KS(+) (Stratagene, San Diego, CA) (called B2.18). The 6kb BglII fragment, which begins just downstream of the ATG initiator codon and extends in the 3' direction, was subcloned into the BamHI site of Bluescript KS(+) (called pmTERTgen-BglII). A preliminary DNA sequence of a portion of B2.18 containing the ATG